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L1	420	handler.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:10
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L8	2673	l6 with promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:11
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L16	3092	ITR or "inverted terminal repeat"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:13
L17	5	l14 and l16	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:15
L18	1	l15 and l16	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:14
L19	12	(deletion or deleting) WITH l3	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:17
L20	18482	drosophila	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:17
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L22	1532	l21 and promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:17
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L24	376	bglII WITH HpaI	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:18

L25	1	l24 and l3	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:18
L26	1	"internal sequence" WITH l3	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:19
L27	1	minimum WITH l3	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/05/24 15:19

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2	US 20040255342 A1	Transgenic flies expressing Abeta42-Iowa
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4	US 20040250302 A1	Transgenic flies expressing Abeta42-dutch
5	US 20040244064 A1	Transgenic flies expressing Abeta42-Flemish
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7	US 20040177388 A1	Methods and compositions for the identification and treatment of neurodegenerative disorders
8	US 20020199216 A1	Use of transposable elements for altering gene expression
9	US 20020173634 A1	Methods and compositions for transposition using minimal segments of the eukaryotic transformation vector piggybac

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1	US 20040161827 A1	Insect p53 tumor suppressor genes and proteins
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4	US 20020173634 A1	Methods and compositions for transposition using minimal segments of the eukaryotic transformation vector piggybac
5	US 20020009751 A1	Drosophila homologues of genes and proteins implicated in metabolism and methods of use
6	US 6762291 B1	Insect p53 tumor suppressor genes and proteins
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9	US 6551825 B1	PiggyBac transposon-based genetic transformation system for insects
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1: Insect Mol Biol. 1999 Nov;8(4):449-57.

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Germline transformation of Drosophila melanogaster with the piggyBac transposon vector.

Handler AM, Harrell RA 2nd.

Center for Medical, Agricultural, and Veterinary Entomology, US Department of Agriculture, Gainesville, FL 32608, USA.
handler@nersp.nerdc.ufl.edu

Germline transformation of Drosophila melanogaster was attempted with the piggyBac gene-transfer system from the cabbage looper moth, Trichoplusia ni. Using a self-regulated transposase helper and a white marked vector, a transformation frequency of 1-3% per fertile G0 was obtained, similar to that previously achieved in the medfly. Use of an hsp70-regulated helper increased this frequency more than eight-fold. Transformation with a vector marked with white and green fluorescent protein (GFP) under polyubiquitin-nuclear localizing sequence regulation yielded seventy G1 transformants which all expressed GFP, but only twenty-seven of these expressed eye pigmentation that would have allowed their selection based on white+ expression. PiggyBac transformation in two distantly related dipteran species and efficient expression of the gfp marker supports the potential use of this system in other dipterans, and perhaps insects in general.

PMID: 10634970 [PubMed - indexed for MEDLINE]

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US 20020173634A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2002/0173634 A1

Fraser, JR. et al. (43) Pub. Date: Nov. 21, 2002

(54) METHODS AND COMPOSITIONS FOR TRANSPOSITION USING MINIMAL SEGMENTS OF THE EUKARYOTIC TRANSFORMATION VECTOR PIGGYBAC

(76) Inventors: **Malcolm J. Fraser JR.**, Granger, IN (US); **Xu Li**, Notre Dame, IN (US); **Teresa Beam**, Columbia City, IN (US); **Aurelie Hua-Van**, Cedex (FR)

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(21) Appl. No.: 10/001,189

(22) Filed: Oct. 30, 2001

Related U.S. Application Data

(60) Provisional application No. 60/244,984, filed on Nov. 1, 2000. Provisional application No. 60/244,677, filed on Oct. 31, 2000.

Publication Classification

(51) Int. Cl.[7] C07H 21/02; C07H 21/04

(52) U.S. Cl. 536/23.1

(57) ABSTRACT

More efficient transfer of genes into host cells or embryos to transform the cells or embryos is facilitated by transposition vectors using the minimal amount of nucleotide sequences in the transposon piggyBac required for gene transfer. The transformed cells or embryos may be developed into transgenic organisms.

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L1         34541 S HANDLER?/AU OR BEAM?/AU OR HUA-VAN?/AU OR LI-XU?/AU OR FRASER
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L4             5 S (MINIMUM OR INTERNAL) (S) L2
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L6            51 S L1 AND L2
L7             5 S L6 AND ((MINIMUM OR INTERNAL (S) PIGGYBAC))
L8             2 DUP REM L7 (3 DUPLICATES REMOVED)
L9        674004 S FLUORESCENT OR FLUORESCENCE
L10        79355 S HEAT (2W) SHOCK
L11          292 S UBIQUITIN (2W) PROMOTER
L12       157408 S DROSOPHILA
L13          292 S L11 (P) L11
L14          108 S BGLII (S) HPAI
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L26            9 DUP REM L25 (7 DUPLICATES REMOVED)
L27            0 S L26 AND (DELET? (5W) SEQUENCE)

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L5 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005037456 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15663772
TITLE: **piggyBac internal** sequences are necessary for efficient transformation of target genomes.
AUTHOR: Li X; Harrell R A; Handler A M; Beam T; Hennessy K; Fraser M J Jr
CORPORATE SOURCE: Department of Biological Sciences, and Center for Tropical Diseases Research and Training, University of Notre Dame, Notre Dame, IN 46556, USA.
CONTRACT NUMBER: AI48561 (NIAID)
SOURCE: Insect molecular biology, (2005 Jan) 14 (1) 17-30.
Journal code: 9303579. ISSN: 0962-1075.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200503
ENTRY DATE: Entered STN: 20050125
Last Updated on STN: 20050325
Entered Medline: 20050324

AB A previously reported **piggyBac** minimal sequence cartridge, which is capable of efficient transposition in embryo interplasmid transposition assays, failed to produce transformants at a significant frequency in Drosophila melanogaster compared with full-length or less extensive **internal** deletion constructs. We have re-examined the importance of these internal domain (ID) sequences for germline transformation using a PCR strategy that effectively adds increasing lengths of ID sequences to each terminus. A series of these piggyBac ID synthetic deletion plasmids containing the 3xP3-ECFP marker gene are compared for germline transformation of D. melanogaster. Our analyses identify a minimal sequence configuration that is sufficient for movement of piggyBac vectored sequences from plasmids into the insect genome. Southern hybridizations confirm the presence of the piggyBac transposon sequences, and insertion site analyses confirm these integrations target TTAA sites. The results verify that ID sequences adjacent to the 5' and 3' terminal repeat domains are crucial for effective germline transformation with piggyBac even though they are not required for excision or interplasmid transposition. Using this information we reconstructed an inverted repeat cartridge, ITR1.1k, and a minimal piggyBac transposon vector, pXL-BacII-ECFP, each of which contains these identified ID sequences in addition to the terminal repeat configuration previously described as essential for mobility. We confirm in independent experiments that these new minimal constructs yield transformation frequencies similar to the control piggyBac vector. Sequencing analyses of our constructs verify the position and the source of a point mutation within the 3' internal repeat sequence of our vectors that has no apparent effect on transformation efficiency.

L5 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001609493 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11683259
TITLE: The **minimum internal** and external sequence requirements for transposition of the eukaryotic transformation vector **piggyBac**.
AUTHOR: Li X; Lobo N; Bauser C A; Fraser M J Jr
CORPORATE SOURCE: Department of Biological Sciences, and Center for Tropical Diseases Research and Training, University of Notre Dame, IN 46556, USA.
CONTRACT NUMBER: AI 40960-01 (NIAID)
SOURCE: Molecular genetics and genomics : MGG, (2001 Oct) 266 (2) 190-8.
Journal code: 101093320. ISSN: 1617-4615.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112

ENTRY DATE: Entered STN: 20011102
Last Updated on STN: 20020123
Entered Medline: 20011204

AB The piggyBac element from Trichoplusia ni is recognized as a useful vector for transgenesis of a wide variety of species. This transposable element is 2472 bp in length, and has a complex repeat configuration consisting of an internal repeat (IR), spacer, and terminal repeat (TR) at both ends, and a single ORF encoding the transposase. Excision assays performed in microinjected T. ni embryos using plasmids deleted for progressively larger portions of the **piggyBac internal** sequence reveal that the 5' and 3' IR, spacer, and TR configuration is sufficient for precise excision of **piggyBac** when transposase is provided in trans. Interplasmid transposition assays using plasmids carrying varying lengths of intervening sequence between the **piggyBac** termini in T. ni demonstrate that a **minimum** of 55 bp of intervening sequence is required for optimal transposition, while lengths less than 40 bp result in a dramatic decrease in transposition frequency. These results suggest that the piggyBac transposase may bind both termini simultaneously before cleavage can occur, and/or that the formation of a transposition complex requires DNA bending between the two termini. Based on these results we constructed a 702-bp cartridge with minimal piggyBac 5' and 3' terminal regions separated by an intervening sequence of optimal length. Interplasmid transposition assays demonstrate that the minimal terminal configuration is sufficient to mediate transposition, and also verify that simply inserting this cartridge into an existing plasmid converts that plasmid into a non-autonomous piggyBac transposon. We also constructed a minimal **piggyBac** vector, pXL-Bac, that contains an **internal** multiple cloning site sequence between the minimal terminal regions. These vectors should greatly facilitate the utilization of the piggyBac transposon in a wide range of hosts.

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